UNPUBLISHED PRELIMINARY DATA

Free Radical Ions

Investigator: R. N. Adams

A study has been initiated of the electrochemistry and optical spectroscopy of aromatic free radical ions. Such studies are fundamental to an understanding of the organic electrode processes in such practical electrochemical devices as fuel cells, regenerative EMF systems, and solar energy conversion devices. Solvent effects on the electronic spectra of nitrobenzene anion radical have been examined and interpreted in light of recent electron paramagnetic resonance data. The results are summarized in the accompanying preprints.

U of Kansas
Oct 15, 1964 - March 30, 1965
NsG-298

Transformation of Inorganic Sulfur Compounds

Investigator: J. M. Akagi

Previously, we reported that the products of pyruvic acid oxidation by Clostridium nigrificans were acetate, carbon dioxide and molecular hydrogen (Akagi, 1964). An exogenous electron transport system was required together with coenzyme A, thiamine diphosphate and a divalent cation.

Recently, we observed that centrifugation of the crude extracts at 105,000 x g for 3-4 hours, to remove particulate fractions, gave a supernatant fraction that oxidized pyruvate to acetyl phosphate without the presence of any exogenous electron carrier. These extracts are currently being studied for their ability to metabolize pyruvic acid.

Present results indicate that extracts of C. nigrificans, (1) do not evolve molecular hydrogen unless an artificial electron carrier is present, (2) some of the electrons derived from pyruvate oxidation are passed to the sulfite-reducing system, forming hydrogen sulfide, (3) in the absence of an artificial electron carrier, acetate, CO_2, lactate and ethanol are produced. These results suggest that the pathway of electrons from pyruvate is not solely to the sulfite-reductase system, but also proceeds to certain enzyme systems such as lactic and alcohol dehydrogenases.

The nature of the electron carrier participating in the reduction of sulfite to H_2S has been partially elucidated. It is a protein that adsorbs tightly to diethylaminoethyl cellulose and is removed by high salt concentrations. This substance is highly labile and inactivates rapidly under conditions that are ideal for most proteins. The carrier transfers electrons from pyruvic dehydrogenase to the sulfite-reductase system. In this respect it is unlike ferredoxin which is found in many anaerobic bacteria. Studies are in progress to determine if other electron transport systems are operating in the process of sulfate-reduction.

Adaptability of Human Operator as a Controller

Investigator: H. Akashi

The ability of human operators to stabilize a control system was investigated by analog simulation for the purpose of finding the adaptive capacity in an emergency such as may be encountered in spacecraft. A variety of operators were asked to perform stable control of integral-type, second and third order systems for various parameter combinations. Limits were given to the manipulating variable to simulate most actual applications. The ratio between the time during which the operator can keep the output within preassigned limits and the time of the test run was taken as the performance index. The result shows that the control ability of an operator is expressible by an asymptotic curve in the parameter plane of time constant and gain. Increase in time constant of the system poses as much difficulty for the operators as the increase in gain constant. Third order systems appear to be the highest possible system an ordinary human controller can operate with reasonable value of the performance index. Trained pilots generally showed larger parameter values for a given index, which fact may be used to evaluate the effect of training quantitatively.

Iron-Copper Magnets

Investigator: N. I. Ananthanarayanan

To date, the following work has been accomplished on the above project:

1. Preparation of iron-copper mixed-crystal oxalates and of iron and copper oxalate mixtures.
2. Copper-coating of iron powders.
3. Calibration of reduction furnace and design of pole-pieces and magnet-coils for high-field permeameter magnet.

Preparation of Iron-Copper Oxalates:

A simple technique of preparing iron-copper oxalates suitable for low-temperature decomposition-reduction into five iron-copper powders has been developed. The technique consists in the precipitation of iron-copper oxalates by reaction between aqueous solutions containing the desired proportions of iron and copper ions and a concentrated solution of oxalic acid. In contrast with the difficulties experienced in co-precipitating iron and copper formates from solutions, the oxalates co-precipitate readily. High yields are easily obtained and the technique is suited for quantity production. Using the technique, one-pound lots of the oxalates with 2.5 and 5 atom percents of copper have been prepared for reducing into iron-copper powders.

Copper Coating of Iron Powders:

For the preparation of permanent magnets from ultrafine iron and other ferromagnetic powders, it is desirable to have a thin non-ferromagnetic coating on the individual particles. Because of the extremely low solid solubility of copper in iron at relatively low ambient temperatures, copper coating of iron powder particles appears to be suitable. Coating standard grades of relatively coarse iron powders by the replacement reaction was successful. For this purpose, the iron particles are treated with a dilute, acidified aqueous solution of a copper salt and the liquid is decanted off. The powder slurry is next washed repeatedly with water, then with alcohol, and then it is dried. The freshly-cemented copper surface tarnishes quickly, thus requiring a low-temperature H_2-reduction

to restore it to its original condition. The experiments are to be continued with ultrafine iron powders.

Furnace Calibration; Design of pole-pieces and magnet coils for high field permeameter:

Required calibrations of the reduction furnace for operation at temperatures below 550° C have been completed.

Pole-pieces and magnet-coils needed to convert the available magnet yoke into a high-field permeameter magnet have also been designed.

Work in Progress

The following work, now in progress, is scheduled for completion by June 1965:

1. Preparation of iron-copper oxalates with up to 25 atom percent copper.
2. X-ray diffraction studies of iron-copper oxalates in order to determine whether or not the copper is in solid solution.
3. H_2-reduction of iron-copper oxalates.
4. X-ray diffraction and electron microscopy of the oxalate-reduced iron-copper powders.
5. Construction of the high-field permeameter.
6. Experiments on the copper coating of ultrafine iron powder.
7. Construction of an electrolytic cell for co-precipitation of iron and copper powders.

Several items of equipment will be procured when they are needed in the progress of the work.

Dispersion Strengthening of Metals

Investigators: M. P. Bauleke and Babu Thakur

The dispersion of small non-metallic particles within a metal is known to improve the high temperature physical properties. Most work has centered around oxide additions; but why only oxides? When using low-melting aluminum, other materials such as carbonates and fluorides should be as usable as oxides. Such knowledge is needed to help design the correct materials for fabrication of component parts of space vehicles.

To date, powdered aluminum test specimens containing 3 and 9 percent additions of calcium carbonate, calcium fluoride and zinc oxide have been hot pressed. Elevated temperature testing and comparison of the various test specimens is proceeding. After hot testing is completed, metallographic studies will be made of the grain structure and of the bonding between metallic and non-metallic phases.

Effect of Nonionizing Radiation on Interferon Production

Investigator: R. H. Bussell

The effects of sublethal doses of ultraviolet irradiation on the ability of cells grown in culture to support virus multiplication and interferon production is being investigated. Interferon is a protein produced by virus-infected cells which is capable of limiting virus multiplication. Thus, interferon may constitute an important host defense mechanism. This investigation should be of value to those concerned with the protection of man in space from various radiation hazards.

Primary chick embryo cells in suspension were exposed to UV. Variations in distance and time were employed. The UV source was a 15 watt Sylvania germicial lamp housed in a parabolic, specular aluminum reflector. At a distance of 34 cm., the minimum lethal dose was between 10 and 15 min.; at 16 cm. it was between 5 and 6 min. Cell viability was determined by the eosin dye exclusion technique. Primary chick embryo cells growing as monolayers were exposed to UV at 16 cm. Cell viability was determined by replating the cells and determining their ability to reform monolayers as compared to non-irradiated control cells. The minimum lethal dose was not determined, but it was much less than that observed for cells in suspension as determined above. Monolayers of McCoy cells (a continuous line of human amnion cells) were exposed to UV under similar conditions and assay methods, and the minimum lethal dose was between 5 and 10 sec.

Standard techniques have been worked out for interferon preparation and assay. Interferon was prepared in chick embryos and assayed in chick embryo cell cultures using a tube dilution method and a bovine enterovirus as the challenge virus.

Studies on the Antigenic Properties and Structure of Isozymes of Schistosoma japonicum and Trichinella spiralis

Investigator: D. G. Dusanic

Basic immunological phenomena associated with resistance to infectious agents are being investigated immunologically and biochemically employing two metazoan human parasites, Schistosoma japonicum and Trichinella spiralis. The principles elucidated by the techniques evolved by this study may be applied to the maintenance of health among future space travelers and population.

At present, no results have been obtained for S. japonicum due to difficulties encountered with the first shipment of snails from the Philippines. All snails arrived dead because of poor packing. A second shipment is in transit.

Somatic extracts (som. ext.) were prepared from T. spiralis larvae homogenized in saline at 0° C and secretion-excretion products (SEP) were collected from larvae incubated at 27° C in saline. Sera were obtained from rabbits and rats bled at various times after infection. Polyacrylamide gel electrophoresis, Ouchterlony immunodiffusion, immunoelectrophoresis, and biochemical assays of enzyme activities were performed on these parasite substances and the homologous sera. Proteins were stained with amidoschwartz stain (A.S.). Isozymes of lactic dehydrogenase (LDH) were localized with sodium lactate substrate and Nitro BT stain. LDH activity was measured spectrophotometrically by the oxidation of reduced DPN. The results of these analyses are shown in Tables 1 and 2.

Table 1

	Number of Fractions Polyacrylamide gel Electrophoresis		Immunodiffusion Precipitin Lines (A.S.)		Immunoelectrophoresis Precipitin Lines Somatic Extract	
	A.S.	LDH	Som. ext.	SEP	A.S.	LDH
T. spiralis larval som. etc.	9-10	2				
" " " SEP	6	1				
Pooled normal rabbit sera	13	5	0	0	0	0
Pooled sera from rabbits infected 2 weeks	*	*	2	0	0	0
Pooled sera from rabbits infected 4 weeks	16	4	5	1	0	0
Pooled sera from rabbits infected 6 weeks	*	*	7	3	4	0
Pooled sera from rabbits infected 8 weeks	16	4	9	3	6	2
Pooled normal rat sera	14	5	0	0	*	*
Pooled sera from rats infected 5 weeks	*	*	7	2-3	*	*
Pooled sera from rats infected 1 year	*	*	7	3	*	*

* Not yet analyzed.

Table 2

	Biochemical assay of LDH activity	
	Units of LDH Activity	Per Cent Inhibition
0.1 ml T. spiralis larval som. ext.	55	
0.1 ml pooled normal rabbit sera	19	
0.1 ml pooled sera from rabbits infected 8 weeks	13	
0.1 ml T. spiralis larval som. ext. + 0.1 ml pooled normal rabbit sera	73	0
0.1 ml T. spiralis larval som. ext. + 0.1 ml pooled sera from rabbits infected 8 weeks	60	12

Polyacrylamide gel electrophoresis demonstrated that LDN isozyme is secreted by the larvae. In the rabbit this results in the production of anti-LDH antibodies which appear in the serum by the eighth week. These antibodies combine with the two LDH isozymes as shown by immunodiffusion and appear to inhibit enzyme activity as indicated by the biochemical assay. During infection other changes in serum isozymes, proteins and precipitating antigen-antibody reactions are observed. Additional studies will be performed with the LDH and other parasite enzyme systems to further determine their importance in the development of a functional immunity and subsequently to characterize their structure.

Telemetering Pulse Rates

Investigator: E. R. Elbel

Securing pulse rates on active subjects has been accomplished satisfactorily by using the telemetering technique.

FM station interference has been eliminated by the selection of a relatively interference-free wavelength and then using one-fourth the wavelength in inches for the aerial for distance transmission.

Various types of electrodes have been used varying from dime-sized cup-like silver discs to relatively expensive commercial electrodes. As satisfactory as the commercial electrodes are those which have been constructed from 5/8" plastic buttons in which a one-fourth diameter in hole has been bored. A thin piece of silver is "sandwiched" between the button and a piece of thin plastic. The bored opening serves as a receptable for electrode paste. The cost is estimated at about 15 cents each.

To apply the electrodes, the skin is thoroughly cleansed and sprayed with an adherent (tuff-skin). The electrode is sealed to a circular adhesive plaster (Kurotex) with collodion. The edges of the plaster are sealed down with collodion. This method prevents perspiration interference and makes it possible to secure satisfactory readings when the subject is emersed in a tank of water.

Qualitative and Quantitative Effects of Various Adjuvants Upon Antibody Synthesis

Investigator: M. J. Freeman

The purpose of this investigation is to elucidate the effects of various immunization schemes, either with or without various adjuvants, upon the total and selective antibody response of the rabbit. Recent information indicates that significant functional differences may exist among the various physicochemical classes of antibody globulins. Systems of immunization which might effectively stimulate either an increased total or selective synthesis of one or more classes of antibody would be of obvious medical value. The nature and importance of pathogenic agents to be encountered during exploration of outer space cannot as yet be effectively evaluated. It is likely, however, that without prior antigenic experience or immunity to such agents, or through physical influences which might weaken his resistance to inherent latent infection, man would be extremely susceptible to debilitating disease. The capability to induce rapid formation of potent antibodies, without undesirable side effects such as hypersensitivity, would be extremely valuable in space exploration.

We have immunized groups of rabbits with a purified antigen alone and with various adjuvants. Several immunochemical techniques including radioimmunoelectrophoresis, hemagglutination, precipitation and immunodiffusion, and others have been or will be applied to determine the composition of the immune sera and to establish the properties of the various antibodies which these contain. Although evaluation is still in progress, preliminary results indicate that significant differences in both the total antibody and the classes of antibody synthesized may be effected by certain immunization schemes.

Microwave Spectroscopic Studies at Elevated Temperatures

Investigator: M. D. Harmony

An extensive search for the microwave absorption spectrum of urea, $(NH_2)_2CO$, has been performed at 125° C using the heated cell described in our previous report. No absorption lines due to urea have yet been identified, although some fifty or more ammonia lines have been observed and catalogued. This dense ammonia spectrum seems to be the main obstacle to the observation of urea absorption lines. We are currently trying other temperature and pressure conditions in order to increase the urea concentration in the gas phase. A flow system using an inert carrier gas will also be tried. This technique will have the advantage that a fresh sample will always be present, and it will also minimize diffusion to, and possible reaction at, the walls of the absorption cell.

The mechanism of the ammonia production is not clear. We suspect that a reaction of the type

$$2(NH_2)_2CO(s) \rightleftharpoons NH_3(g) + (NH_2)_2NH(CO)_2(s)$$

is occurring in addition to the vaporization of urea. We are investigating the thermodynamics of this and other possible chemical decompositions.

Regulation of Metabolic Pathways in Chlorella

Investigator: P. A. Kitos

Prolonged journeys in space by man will not be possible without a continuous supply of molecular oxygen. Unicellular plants such as chlorella may be used not only to provide the necessary oxygen but also to regenerate dietary essentials from some of man's own metabolic products. Using only the energy of light, the plants produce oxygen and purify the air of respiratory carbon dioxide.

In the absence of light, plants, like animals, consume reserve sugars, oxidizing them to carbon dioxide and water. However, in the presence of light, plants produce molecular oxygen by the "photolysis" of water. At the same time they consume some oxygen by the process of respiration. The rate of respiration during active photosynthesis is equal to that which occurs in the dark, but because of the overwhelming production of oxygen the consumption can be measured in the light only with difficulty.

In order to determine whether the respiration in the light and dark involve use of the same substrates, a series of experiments with chlorella pyrenoidosa using 1-^{14}C-glucose were carried out. If this substrate were used for respiratory purposes in the light, much of the ^{14}C would be converted to carbon dioxide and refixed into the storage polysaccharides. The radioactive label would be redistributed throughout the carbon skeleton of the hexose subunits.

The polysaccharide of chlorella cells was isolated, purified and hydrolyzed to glucose. The glucose was degraded, carbon by carbon, in order to determine the distribution of radioactivity. Results of these experiments have established that no more than 5 percent of the incorporated radioactive label is randomized in the storage polysaccharide. Based on these and other data, we find that the organism uses only one-fourth to one-sixth as much glucose for respiration in the light as in the dark.

Cell Growth

Investigator: F. E. Leaders, Jr.

The original proposal was divided into four sections. Progress, as previously, is reported by sections.

1. Cell culture techniques.
2. Chick chorio-allantoic membrane:
   Both sections completed and reported April 10, 1964.
3. Antigenicity.

Sheep ESF obtained from Armour and Co. (National Heart Institute, Hematology Study Section) and rabbit ESF prepared in our laboratory were studied. A modification of the Gel-diffusion method described by Robbins, et al. was used. There was no evidence that sheep and rabbit ESF are antigenically related. The control antigens and rabbit ESF all reacted strongly with anti-rabbit ESF guinea pig serum but not at all with anti-sheep ESF rabbit or guinea pig serum. It was possible to distinguish differences in number and position of lines formed by each system. Sheep ESF did not react with anti-rabbit ESF guinea pig serum. There was no reaction between preimmune serum and the antigens tested.

Human ESF extracts and sheep ESF extracts were analyzed by immunodiffusion techniques in conjunction with Dr. William Larson, immunohematologist, Department of Medicine. Sheep ESF demonstrated an electrophoretic pattern which contained a faint protein zone in the albumin through α globulin range while the human ESF extracts contained albumin, some α_1 and gamma globulin and a pronounced α_2 globulin.

Ouchterlony precipitin tests have been performed with goat anti-human and goat anti-Bence Jones protein anti-serum and human ESF as the antigen. A positive reaction was obtained between human ESF and goat anti-human anti-serum, but with serum from no other specie. The possibility that goats may not have the protein material normally associated with ESF is raised. This may be studied further if time permits before this grant expires. Human ESF is not antigenically similar to Bence Jones protein.

4. Effects on Leukemia

The effect of ESF on induction and progress of leukemia in anexic, clean (exanexic) and conventional mice is being tested. On June 15, 1964

ESF was injected into groups of clean and conventional animals in a single dose of 50 or 100 units/kg. This was either alone or combined with an injection of a 20% suspension of minced splenic tissue from leukemic mice. These animals were observed for signs of leukemia (spleen size, lymph nodes, etc.) and deaths due to leukemia. This experiment is still in progress. The data obtained to date is as follows:

Conventional animals receiving ESF plus leukemia extract - 12 deaths due to leukemia/40 animals. Conv. animals, ESF only - 6 deaths due to leukemia/40 animals. Conv. controls 2/20. Clean animals, ESF + leukemia extract - 1/40. Clean animals, ESF only - 0/40. Clean controls 0?20. Clean - leukemia extract only 3/20. Clean animals - inactivated ESF only - 0/20.

These data suggest enhancement by ESF of the ability of the leukemia extract to induce leukemia but does not suggest a primary induction by ESF alone. To eliminate the possibility that the enhancing effect could be due to viral contamination, attempts were made to culture them from the ESF extracts used. No virus activity could be demonstrated.

5. Funds from this grant are also being used in partial support of an additional project. Increased ESF levels have been reported to be associated with different types of cancer in humans and tumors in animals. Many forms of cancer in many species of animals are known to be caused by viruses. The possibility that an ESF-like compound might be involved in the relationship between tumor and virus is being tested. Preliminary experiments indicate such an involvement.

Experimental Study of the Effect of Plastic Deformation on the Yield Condition of Metals

Investigator: L. E. Linzell

McComb at Langley Research Center, Osgood at the National Bureau of Standards, Naghdi, et. al. and others have studied the first subsequent yield surface for metals that have been loaded radially into the plastic range. Some have determined additional yield surfaces caused by further plastic loading with the same stress ratios. The second subsequent yield condition resulting from a second plastic load, but with different stress ratios, is the subject of this investigation.

Two sets of experiments were conducted on eight thin walled tubes subjected to internal pressure and axial tension or compression. Test "A" was to establish and determine the first subsequent yield surface by loading the annealed tubular specimens to $2\sigma yp$ (twice the initial tensile yield point) in simple tension in the axial direction. Test "B" established a first subsequent yield surface by loading annealed specimens to $2\sigma y$ in simple tension in the circumferential direction, unload, and then establish and determine the second subsequent yield surface produced by loading to $2\sigma yp$ in the axial direction. Thus test "B" is the same as test "A" except that it was preceded by a plastic loading in circumferential tension.

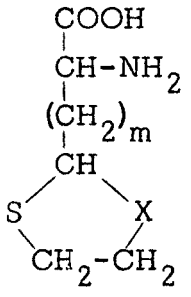
The interpretation of the results of these experiments is continuing at the present but tentative results indicate that the first subsequent yield surface for test "A" and the second subsequent yield surface for test "B" are practically the same. This means that in test "B" the effects produced by the circumferential tension load were "erased" by the subsequent plastic loading in axial tension.

Dithiolanes as Radioprotective Agents

Investigator: M. P. Mertes

The effect of ionizing radiation on life and the subsequent lethal damage has stimulated research in the synthesis of organic compounds that may prove useful for internal chemical protection against high energy radiation such as that encountered in space. The toxicity of the known protective agents has hampered their effective application in space medicine.

A possible approach to chemical protection, being examined in this project, is to modify the target molecules in the cells by non-toxic agents containing radiosensitive sulfide groups. The compounds that have been synthesized in this project are oxothiolane and dithiolane substituted amino acids. The synthesis of the indicated analogs of compound I is now in progress.



X= S or O

m= 0, 1, 2

When sufficient quantities are available biological testing will be done by comparing survival time after exposure of treated and control mice to lethal doses of X-radiation.

Research on Cavitation

Investigator: R. B. Mesler

The effect of proximal rigid boundaries on the motion and damage capabilities of spark bubbles is investigated by means of a qualitative photographic study. It is determined that an axially symmetric bubble collapsing nonhemispherically against a solid boundary can form a liquid jet which impinges against the solid boundary, but the jet has little or no damage capability. Observations of the toroidal minimum volume and associated damage to the boundary in the form of circular dents on indium indicate a pressure pulse damage mechanism.

A paper entitled "A Photographic Study of the Dynamics and Damage Capabilities of Bubbles Collapsing Near Solid Boundaries," by N. D. Shutler and R. B. Mesler was presented at the winter meeting of the ASME in New York. (Paper No. 64-WA/FE-13)

Leidenfrost Phenomenon

Investigator: R. B. Mesler

When liquid drops are placed on a very hot surface the drops will dance around and exist for a surprisingly long time. As the drop moves across a surface there is heat transfer from the surface to the drop. This investigation is directed toward measuring the transient surface temperature beneath such a drop in an effort to calculate the transient heat flux to the drop.

High speed motion pictures of drops moving across fast response surface thermocouples have been obtained. In these pictures the surface temperature response has been photographically recorded superimposed on the film. An analysis of the photographs has been started.

In analyzing the high-speed motion picture films we have observed that the drop behaves differently than it appeared to act by direct observation. In particular, drops were observed to splatter in a manner that was not apparent before.

The importance of studying the heat transfer to drops in this manner is that this will provide fundamental information necessary for a better understanding of film boiling.

Cooperative Studies with Erythropoietin (ESF)

Investigator: M. P. Mohn

Several conditions which are known to stimulate production of ESF in humans and animals are also involved in both conventional high altitude flight and in space flight (e.g., exposure to altitude or reduction in the partial pressure of oxygen). Detailed knowledge of ESF and its biological effects are, therefore, significant to space research. In this project, three aspects of ESF have been undertaken for study.

General: Large rabbits have been used to provide serum for the preparation of ESF, utilizing the method of Borsook. To date, approximately 330 ml of ESF have been purified, bioassayed, and utilized for various experiments. In addition, approximately 125 ml are currently ready for bioassay. Two batches of ESF, totaling 550 ml, proved unsatisfactory for use in experiments.

I. Isolation, Purification and Identification of ESF from Fractionated Human Blood (F. E. Leaders and A. A. Werder): The four plasma fractions cited in the grant application have been extracted according to the method of Borsook, and biological assays were performed utilizing the starved rat-Fe59 uptake method. Some ESF activity was present in all four fractions, but the Fe59 uptake of the experimental animals was not statistically different from that of control animals. Consequently, human plasma fractions appear to offer no advantage over anemic laboratory animals as an experimental source of ESF. Work on this portion of the project has been terminated.

II. Studies of ESF in Relation to the Integument and Other Epithelial Tissues (M. P. Mohn). Injection of ESF or Cobalt in young underfed rats had no effect on nail growth (mm/day) or hair growth (both spontaneous replacement and growth induced by plucking). However, recent experiments with old rats and with rats injected with cortisone disclosed faster nail growth in ESF and Cobalt treated animals, but little if any effect on hair growth. These experiments are currently being repeated, and related studies have been scheduled.

Preliminary studies of the effects of ESF on mitotic activity in the integument and other tissues are inconclusive, and additional experiments have been planned.

Studies of wound healing in underfed young rats indicate enhanced healing with ESF treatment, and further studies are in progress including transplantation experiments.

III. A Study of the Effect of ESF on Antibody Formation and Morphology of Immunologically Competent Cells (A. L. Chapman and O. J. Mira). In control studies, rats were injected with Salmonella typhosa H antigen and tissues removed for light and electron microscopic evaluation after 6 days. As expected, the number of plasma cells was elevated, but more electron micrographs are needed to evaluate any cellular changes within plasma cells and other related cell types. Animals will soon be injected with ESF (with or without the antigen) and tissues removed for comparison with the appropriate stage of normal immunological response.

Scattering of Waves from a Rough Surface

Investigator: R. K. Moore

An exact theory has been developed for scattering of waves from a rough surface defined by a differentiable function. Green's second theorem is used and a Dirichlet or Neumann problem is assumed. This leads to a Fredholm integral equation of the first kind which is solvable with a known complete set of functions onthogonal over the area of illumination.

The above theory can be used to check the approximate methods developed by other authors and apply to problems where no approximation can be made.

The frequency dependence of the statistical parameters of the surface is being investigated. This will clarify the meaning of the parameter values obtained through fitting the experimental mean power return curves.

A paper has been prepared with support from this project and will be published on March 15, 1965 in the Journal of Geophysical Research.

Simulation of DME-VOR Antenna Siting

Investigator: R. K. Moore

The basic technique of simulating radiation from a UHF radio-navigation aid using acoustic waves in water was investigated and found practical under certain circumstances. The effect of scattering objects, such as buildings, near the antenna was checked for a very simple situation, and the modeling appeared satisfactory. Unfortunately, only buildings within the immediate vicinity (say a radius of a mile) can be conveniently used in the existing modeling facility. The problem of developing a transducer with a suitable radiation pattern was also studied experimentally. The transducer developed had a pattern moderately uniform over about 240°; considering the short time available to develop the transducer, this is very encouraging.

Catalysis of Silicone Decomposition

Investigators: R. L. Schowen and K. Latham, Jr.

In contrast to previous indications, the methanolysis of aryloxytriphenylsilanes is found to be general-base catalyzed with Brönsted $\beta = 0.71$ (p-methoxyphenoxy), 0.73 (phenoxy) and 0.77 (p chlorophenoxy). Thus the binding of the base in the transition state increases from about 70% to about 80% with an increase in electron withdrawal in the aryl moiety. The rates for various aryl compounds do not correlate with the Hammett substituent constants, but free energies of activation are linear in free energies of ionization of the corresponding phenols in methanol, as determined in this laboratory. The slopes of these relations are greater than unity, apparently due to d-orbital withdrawal of oxygen lone-pair electrons by silicon in the reactant molecule. Application of the reacting bond rule of Swain and Thornton indicates the mechanism of catalysis to be "inverse classical" general-base (lyate ion and conjugate acid) catalysis.

Catalysis of Silane Decomposition

Investigators: R. L. Schowen and K. O'Donnell

Analytical methods, the development of which was described in the last report, have been applied to the measurement of catalytic reaction rates of the methanolysis of triphenylsilane at 25°. The reaction is either specific-catalyzed or general-base catalyzed with Brönsted $\beta > 0.8$. Thus the base is more than 80% bound at the transition state. Salt effects have been examined and shown to account for the apparent uncatalyzed reaction, as indicated previously. Rates in phenoxide-phenol and cresoxide-cresol buffers have been examined over a wide concentration range; rates in acetate-buffers are now under study. Acidities of a variety of phenols in methanol have been determined.

Solutions of Non-Linear Differential Equations by Approximate Methods

Investigator: S. M. Shah

Non-linear differential equations for which exact solutions cannot be obtained occur frequently in engineering and physical science problems. In this project we are investigating three approaches to this obstacle:

1) the utilization of continued-fractions theory to obtain approximants for solutions of such equations

2) the study of singularity-free regions of second-order non-linear equations

3) the use of difference-equations methods

We have considered the differential equation

$$\text{(A)} \qquad \frac{dw}{dz} = f(w,z)$$

where $f(w,z)$ is holomorphic for $|z|<r_1$ and holomorphic for $|w|<r_2$. We prove the following: Let $u_1(z)$ and $u_2(z)$ be two different holomorphic solutions of (A) for $|z|<r_1$; then either

(1) $w(z) = u_1(z) + c\left[u_2(z) - u_1(z)\right]$ for $|r|<r_1$, where c is a constant ($\neq 0, \neq 1$) and $f(w,z)$ must be linear in w, or

(2) there exists a holomorphic function $F(z)$ for $|z|<r_1$ such that

$$w(z) = u_1(z) + F(z)\left[u_2(z) - u_1(z)\right]$$

and $F(z) \neq 0, \neq 1$ for $|z|<r_1$.

In the case (2), there exists a constant $L = L(F)$ such that $r_1 \leq L$.

The converse of case (1) is also true: i.e., if $f(w,z)$ is linear in w, and $u_1(z)$ and $u_2(z)$ are two different holomorphic solutions of (A), then any other analytic solution $w(z)$ of (A) can be written as

$$w(z) = u_1(z) + c\left[u_2(z) - u_1(z)\right],$$

where c ($\neq 0, \neq 1$) is a constant.

Let $p(z)$ be regular in a simply-connected domain D and let $w(z)$ be a solution of

$$\frac{d^2w}{dz^2} + p(z)w(z) = 0$$

such that $w(a) = w(b)$ for $a \in D$, $b \in D$, $a \neq b$. Then

$$w(\zeta) = w(a) + \int_C g(z, \zeta)p(z)w(z)dz$$

where $g(z, \zeta)$ is the Green's function of the system $w''(z) = 0$, $w(a) = w(b) = 0$, and where C is a rectifiable Jordan arc connecting a and b in D with ζ as an interior point of C.

If D is convex, then

$$\sqrt{\frac{6}{K}\left(1 - \frac{|w(a)|}{M}\right)} \leq R = |a - b|$$

where $K = \max_{0 \leq r \leq R} |p(a + re^{ie})|$, $e = \arg(b - a)$, and $M = \max_{0 \leq r \leq R} |w(a + re^{ie})|$.

Let $g(z) = \sum_{p=1}^{\infty} b_p z^p$ be a proper entire function with maximum term $\mu(r) = \max\{|b_p| r^p\}$ for $|z| = r$. Let $e^{g(z)} = \sum_{p=0}^{\infty} a_p z^p$ and let r_n be the radius of the largest circle with center at $z = 0$ whose interior contains no zero of the n^{th} section, $s_n = \sum_{p=0}^{\infty} a_p z^p$.

The lower order λ of $g(z)$ is defined by

$$\lambda = \underline{\lim} \quad \frac{\log \log \mu(r)}{\log r},$$

and let β_n be such that $\mu(\beta_n) = n$. Then it has been proved that

$$\lambda = \underline{\lim} \quad \frac{\log \log n}{\log \beta_n}.$$

If $\lambda < \infty$, then $\log \beta \sim \log r_n$ and hence

$$\lambda = \underline{\lim} \quad \frac{\log \log n}{\log r_n}.$$

Genetic and Biochemical Studies of Azotobacter Species

Investigator: D. M. Shankel

A procedure has been developed for the production and isolation of auxotrophic mutants of the microbial genus Azotobacter. The procedure involves treatment of the cells with 4.4 percent phenol, followed by treatment with a mutagenic agent such as ultraviolet light or n-methyl-n-nitrose-nitrosoguanidine. This technique has now yielded a number of different auxotrophic mutants requiring amino acids or B vitamins for growth. The mutants, individually, require: vitamin B_{12} (two), aspartic acid, glutamic acid, histidine (two), cystine and arginine. In addition, our procedure has been used to produce mutants of algae (C. Van Baalen, personal communication).

The mutant that requires vitamin B_{12} appears to produce steroid materials in significant amounts. Four different chemical tests for steroids yield positive results with this mutant and are negative when run on a large number of other microbial species. Further confirmation by such means as gas chromatography is necessary.

Currently, we are also investigating the utilization of carbon compounds by this microorganism to establish the simplest carbon source that the organism can utilize for growth. Preliminary results indicate that utilization of carbon dioxide occurs under proper conditions of light, temperature and aeration.

Central Recording of Field Biological Data

Investigators: H. W. Shirer and H. S. Fitch

All measuring processes disturb the measured subject. When measuring processes become a significant part of an ecosystem, natural of artificial, organism behavior becomes ambiguous; responses to the environment being, in part, responses to the monitors. Central recording of ecological data via wire transmission lines and radio frequency links is being studied on the University of Kansas Natural History Reservation in order to obtain a maximum quantity of information with minimum disturbance. Four substations, placed in unique environment areas (upper grassland, steep wooded slope, pond area, and valley floor), are connected by multiconductor cable to the central recording laboratory. Pending completion of automatic scanning and recording equipment, incoming data is read manually at the central cable termination.

Attempts to follow temperatures of hibernating snakes in the upper grassland and wooded slope outcroppings failed because the wire leads from the implanted sensors were severed soon after the animals' release. The white footed mouse (Peromyscus leucopus) was a most likely causative agent. Test wires, insulated with either vinyl plastic or Nyclad enamel, with and without repellant (tetramethylthiuram disulfide), were attacked indiscriminately when exposed in the same regions.

Temperatures from six hibernating western box turtles (Terrapene ornata) are being successfully followed via wire lines in the valley floor region. Thermistors, with two meter leads, were cemented to the mid ventral, anterior dorsal, and posterior dorsal surfaces and the animals released. After the animals had burrowed into the soil at sites of their choice, the emerging leads were connected through the local substation to central monitoring panel. Preliminary analysis of the data obtained so far indicate: (1) the depth and position of the animals can be estimated from the temperature levels and gradients, (2) the animals reached depths where temperature variations reflect seasonal patterns in weather variation but do not fall below freezing, and (3) temperature gradients of 1 to 5° F, ventral surface warm, posterior dorsal surface cold, suggest that most

of the animals are in a slightly head down position. Survival, emergence cue information, and depth/position confirmation await spring emergence.

One portable direction-finding receiver and one prototype radio beacon collar for tracking marmots have been completed and tested. Beacon life of four weeks and reliable location from distances of over 1500 feet have been obtained.

Design of a radio location grid for continuous monitoring of position, behavioral, and physiological data from small vertebrates has been started.

Improved Multiple-Layer Insulation for Cryogen Storage

Investigator: G. W. Swift

A technique has been developed for bonding fiber glass strands to a cryogen storage container. The fiber glass strands, precoated with an epoxy resin, are wound on the container at a predetermined helix angle and spacing to produce a network of strands on the container. At predetermined intervals in the buildup of the network, a layer of aluminum foil is wrapped over the outermost strands of the network. As this procedure is repeated over and over, an insulation matrix is formed which consists of multiple aluminum foil radiation shields which are spaced from each other by the low conductivity fiberglass network. We feel that this matrix constitutes an insulating material of the type referred to in current literature as "super" insulation and that it offers several advantages over the other super insulation types. First, the insulation we are developing should have excellent compressive loading characteristics; its aluminum foil shields, unlike those in presently existing multiple-layer blankets, should never touch under compression. Second, acceleration loading should cause no problems for the same reason. Third, the regular pattern in the spacer network in our insulation will be much more permeable to gas flow so that less time will be required for evacuation. Fourth, this matrix may be machineable since it is bonded and the layers are of precise thickness. If so, mitered end sections can be fabricated for the container without causing thermal short-circuiting.

The project was initiated in September 1964 and as of this date we have developed an operational winding apparatus for fabricating our insulation. This apparatus has the following capabilities:

(1) Winds at helix angles to 45°.

(2) Handles either fiberglass strand or roving with diameters from 0.009 to 0.125 inches.

(3) Spaces the fiberglass at distances as small as 0.050 inches.

(4) Handles either epoxy or polyester bonding material.

(5) Handles containers up to three feet in length with diameters from one to four inches.

(6) Winds insulation matrices from one to seven inches in thickness, depending on the internal diameter of the container.

(7) Produces up to 25 radiation shields per inch of insulation matrix depending on size of strand used.

We are presently fabricating a calorimeter for testing the insulation we fabricate. In essence, our design calls for a can of liquid nitrogen to be placed in a cylinder of our insulation. This assembly will be attached to a head flange which fits into the working space of an A. D. Little helium liquefier. By utilizing the Little equipment, we have immediate capability for vacuum to 10^{-5} mm Hg and an external liquid nitrogen shield. Appropriate radiation and conduction guards are placed on the can-insulation assembly. The outer surface of the insulation is held at a predetermined temperature using electric heaters monitored by a thermopile. The boil-off from the can of liquid nitrogen is measured to give q and all variables are determined to measure the effective thermal conductivity, $\bar{k}$, of the insulation matrix. Relative measurements will also be conducted, using Linde SI insulation in place of our insulation matrix to preclude errors in calorimeter design. Because of the availability of the Little machine in our laboratory, the calorimeter construction time will be minimized...we expect to be ready to test insulation samples in late April 1965.

Genetic and Environmental Factors Involved in Streptococcal Disease

Investigator: C. P. Sword

Methods for cell lysis and DNA extraction have been developed with Lancefield group H strains as a model system. A transformation-promoting principle has been found in supernatant fluids from competent cultures. This material induces transformation in transformable strains but has no effect on group A streptococci. Cell walls of group A strains have been treated with penicillin and with a phage-induced muralysin in attempts to transform these strains. At present the group A pathogenic strains have not been transformed.

Collapse Load of Laterally Loaded Thin Rectangular Plates

Investigator: N. Willems

The investigations so far have made clear that to establish the collapse load of laterally loaded thin rectangular plates, the boundary conditions are most important. No difficulty was experienced in realizing fixed boundaries. The ensuing stress pattern in this case developed very rapidly in a membrane type and load capacities are almost unlimited.

The major problem proved to be to realize simple supports. The observed development of high compressive stresses parallel to the supporting edges required very close control of the opening through which the plate was allowed to slide thereby ensuring free lateral movement and at the same time free rotation. These conditions were met by the use of adjustable rollers. Under loading membrane stresses developed in the center of the plate (tensile) and along the edges (compressive) these membrane stresses are accompanied by bending occurring mainly between the central membrane stressed section and the edges. Under progressive loading no definite yield pattern as predicted for thicker plates with smaller deflections did occur except at the four corners. The limiting strength of the plate in all cases proved to be the compressive stability along the edges. Several steel plates of various thicknesses were stress coated and loaded to failure. Following these preliminary tests one plate was strain gaged and loaded up to buckling. At present these results are being analyzed.

Preliminary conclusions at this stage are that simply supported plates under increased loading and large deflections will either act as fixed plates or alternatively will fail under buckling stresses along the edges and no definite yield collapse pattern will develop.

Programmed Sleep Studies: I. Susceptibility to Induction of Sleep

Investigator: E. Wright

Control of the asleep-awake states of man, under conditions of sustained stress, may significantly enhance the effectiveness and well-being of the individual who must function in isolation or semi-isolation circumstances. Considerable variation exists in the individual readiness for sleep induction and the re-instatement of alertness. There seems to be a clear relevance of programmed sleep for individuals who will be engaged in space missions which will have any significant time duration. The determination of the operational conditions and the techniques which would optimize the individual programming of sleep and awake states is the goal of these investigations.

The initial study is concerned with the development (1) of psychodiagnostic sleep behavior instruments; (2) reliable and valid psychophysiological indices which differentiate awake, resting and sleep states; (3) and preliminary testing of several sleep-induction procedures.

The project was given an initial NASA grant in September, 1964. A Sleep Diary and Sleep Inventory were devised, tested and the revised Second Forms are ready for further pilot study. Isolation of low-sleep readiness and high-sleep readiness groups will facilitate the development of the psychophysiological sleep indices. After the basic measures have been developed, considerable inter-disciplinary help will be necessary to help design instrumentation, to collect and analyze data, and to engineer the stress-producing situations for the experimental populations.

Support from NASA and other research sources will be necessary for the basic probes and the full scale study of this area.

REPORTS AND PUBLICATIONS RESULTING FROM NsG-298

Wilder, Martin; Valentine, R. C.; Akagi, J. M. Ferredoxin of Clostridium Thermosaccharolyticum, Reprinted from: Journal of Bacteriology, Vol. 86, No. 4, pp. 861-865, October 1963.

Doneson, Ira N.; Shankel, Delbert M. Mutational Synergism Between Radiations and Methylated Pruines in Escherichia Coli, Reprinted from: Journal of Bacteriology, Vol. 87, No. 1, pp. 61-67, January 1964.

Fung, A. K. Theory of Radar Scatter from Rough Surfaces, Bistatic and Monostatic, with Application to Lunar Radar Returns, Reprinted from: Journal of Geophysical Research, Vol. 69, No. 6, March 15, 1964.

Roofe, Paul G. Effects of Sudden Freezing of Whole Animals (Small Mammals) upon Vascular Patterns, Reprinted from: Anatomical Record, Vol. 148.

Fung, A. K.; Moore, R. K. Effects of Structure Size on Moon and Earth Radar Returns at Various Angles, Reprint from: Journal of Geophysical Research, Vol. 69, No. 6, March 15, 1964.

Dahl, N. A.; Balfour, W. M. Prolonged Anoxic Survival Due to Anoxia Pre-exposure: Brain ATP, Lactate, and Pyruvate, Reprinted from: American Journal of Physiology, Vol. 207, No. 2, August 1964.

Akagi, J. M. Phosphoroclastic Reaction of Clostridium Nigrificans, Reprint - Journal of Bacteriology, Vol. 88, No. 3, pp. 813-814, Sept. 1964.

Buller, C. S.; Akagi, J. M. Hydrogenase of Coleman's Sulfate-Reducing Bacterium, Reprint - Journal of Bacteriology, Vol. 88, No. 2, pp. 440-443.

Clark, W. O. Associative Retrieval by Dynamic Transforms, Presented October 16, 1964 at Southeastern Regional Meeting of Association for Computing Machinery and Southeastern Simulation Council, Atlanta, Georgia.

Magnus, K. Rotational Motions of Satellites in a Radial Gravity Field.

Leaders, Floyd E. et. al., Erythropoietic Stimulating Factor (ESF) as a Stimulant of Cell Growth in vitro, Proceedings of the Society for Experimental Biology and Medicine, CXV, pp. 658-660.

Shutler, N. D.; Mesler, R. B. A Photographic Study of the Dynamics and Damage Capabilities of Bubbles Collapsing Near Solid Boundaries, Presented at Winter Meeting of ASME in New York.

Chambers, J. Q.; Adams, R. N. Solvent Effects on the Visible Spectra of Nitrobenzene Anion Radicals, to be published in Molecular Physics (international journal).